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SUPPRESSION OF ANALGESIA IN RATS INDUCED BY MORPHINE OR L-NAME BUT NOT BOTH DRUGS BY MICROTESLA, FREQUENCY-MODULATED MAGNETIC FIELDS

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In Experiment I, the nociceptive threshold for a criterion response to thermal stimuli (hot plate) for rats was obtained following injections with one of various dosages of either morphine or the nitric oxide synthase inhibitor, L-NAME. On the bases of these results, rats in Experiment II were injected with either morphine (4 mg/kg), L-NAME (50 mg/kg), both morphine and L-NAME, or saline after baseline measurements for nociception and then exposed to either a 1 microTesla magnetic field or to a sham field for 30 min. The magnetic field, presented once every 4 sec, was a frequency-modulated pattern whose pixel durations, for each of the 837 successive values, were 1 msec. Nociceptive thresholds were measured immediately after the exposure and 30 min later. The results indicated that exposure to this magnetic field abolished the analgesic effects of morphine or L-NAME when injected separately but not when injected together relative to rats that had received these drugs and had been exposed to the sham field.

Keywords: Magnetic fields; Morphine; Analgesia; Nitric oxide; MicroTesla; Frequency-modulation; Synergism; Thermal stimuli

Acute exposures to extremely low frequency magnetic fields affect responses to painful stimuli. Kavaliers, Choleris, Prato and Ossenkopp (1998) and Kavaliers, Ossenkopp, Prato and Carlson (1994) found that exposures to 0.5 Hz rotating magnetic fields or 60 Hz magnetic

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fields enhanced nociception. On the other hand, antinociception (analgesia) in response to electrical and thermal stimuli has been reported after organisms were exposed to complex pulsed magnetic fields (Fleming, Persinger and Koren, 1994; Thomas, Kavaliers, Prato and Ossenkopp, 1997; 1998).

Both the alkaloid opioid morphine and the inhibitor of nitric oxide synthase, N^G-Nitro-L-Arginine Methyl Ester (L-NAME), have strong analgesic properties when injected singly or in combination (Ferreira, Santos and Calixto, 1999; Machelska, Labuz, Przelwocki and Przelwocka, 1997; Guney, Gorgan, Tunctan, Uludag, Hodoglugil, Abacioglu and Zengil, 1998). The degree to which temporally complex magnetic fields and analgesics might interact synergistically has significant implication for both the pharmaceutical industry as well the development of new technologies for employing pharmacological agents in combination with the appropriate, temporally-structured patterns of magnetic fields in order to alleviate clinical pain. The present study was designed to investigate the effects of exposure to a complex pulsed magnetic field, when presented alone or in combination with the traditional pharmacological agents morphine and L-NAME, upon rats' latencies of response to thermal stimuli.

METHOD

Subjects

In two separate experiments a total of 86, 60 to 70 day old Wistar albino rats (66 males and 20 females) served as subjects. They had been obtained from Charles River (Quebec) Laboratories. All rats were housed in plastic shoebox cages and were maintained under standard colony conditions with food and water available *ad libitum*. All rats were tested during the middle of the photophase which began at 0800 local time.

General Procedure

On two successive days each rat was removed from the home cage, brought to the experimental room and placed on a hotplate

(Omnitech) maintained at 55°C. At the same time, a foot pedal was depressed to start an electronic timing device. Once a rat was observed to lick two back paws in any combination it was immediately removed from the chamber, the foot pedal was released, and the time was recorded. Any animal that remained on the hot-plate for 90 sec without licking two back paws was removed from the apparatus and assigned this value. The rat was then administered one of the drug treatments subcutaneously and returned to the home cage or taken to a separate room containing the magnetic field generation equipment. All animals were re-tested at 30 min. and 60 min. post-injection.

For Block 1 of Experiment I 20 rats were used to establish the dose dependent analgesic potency of L-NAME upon the criterion response. Rats were randomly assigned (5 per condition) to receive either saline, or 25 mg/kg, 50 mg/kg or 75 mg/kg of L-NAME, following the procedure described above. For Block 2 of Experiment I 20 rats were employed to examine the relationship between response latency and dosage of morphine. Subjects for this experiment were randomly assigned to one of four treatment groups (5 rats per group): saline, or 1 mg/kg, 4 mg/kg or 8 mg/kg of morphine sulphate. The experimental design for both blocks was a $3 \times 2 \times 4$ three-way analysis of variance with two levels repeated (three trials and two days) and one level not repeated (four drug dosages).

For Experiment II, 46 rats served as subjects to investigate the combined effects of morphine, L-NAME and exposure to a complex pulsed magnetic field. Equal numbers of male and female rats were randomly assigned to one of four drug treatment groups: Saline, 50 mg/kg of L-NAME, 4 mg/kg of morphine, or 50 mg/kg L-NAME plus 4 mg/kg morphine. These dosages were selected on the bases of the results of Experiment I. They were effective in elevating response latencies (analgesia) without saturating the measurements (90 sec exposure to thermal stimuli).

Within each of the four drug treatments, half the numbers of animals were exposed to a complex pulsed magnetic field while the other rats were exposed to a sham field. After a 30 min exposure the rats were re-tested, returned to their home cages and then tested again 60 min after the injection (30 min since the removal from the sham-field or magnetic field). The experimental design was a $3 \times 2 \times 2 \times 4$ four-way analysis of variance with two levels repeated. The two within

subject variables were the two days and the three trials (within each day). The independent variables were the four drug treatments and the two magnetic field conditions.

The rats that received the magnetic field treatments were placed in pairs in a 17 cm by 30 cm by 13 cm (deep) plastic cage. The magnetic field (the same equipment employed by Fleming *et al.*, 1994) was generated by two solenoids (each constructed from a 25 cm long by 1 cm diameter soft iron nail wrapped with 1050 turns of 20 gauge insulated wire) whose points were separated by 20 cm. An IBM-compatible personal computer, that was connected to the solenoids through a differential (d.c. coupled) amplifier, was used to generate the complex magnetic field patterns. The Thomas pattern (Thomas *et al.*, 1997), a frequency modulated complex field, was selected as the wave form. It was composed of 837 successive temporal points each of which was presented for 1 ms. During the 30 min of exposure, each presentation of the pattern was separated by 4 sec. Consequently, the rat received 12 presentations per min and 360 presentations per 30 min of exposure.

When applied mechanically by electric current to the footpads this temporal arrangement (1 second on, 4 seconds off), has previously been shown to induce an endogenous opioid mediated analgesia in rodents (Lewis, Cannon and Liebeskind, 1980). The rats were exposed to the complex pulsed magnetic field, or to a sham field (power supply to the solenoids disconnected) for 30 min because this duration had been shown most effective in previous research (Thomas *et al.*, 1998). The magnetic field intensity, measured at the center of the cage peaked at 5 microT. All analyses involved SPSS software on a VAX 4000 computer. Subsequent *post hoc* analyses (SNK and selected correlated *t*-tests) were completed to discern the source of the effect. For all significance tests alpha was set at 0.05.

RESULTS

Three-way analysis of variance demonstrated a significant difference in response latencies for the groups that had received the injections of L-NAME ($F[3, 16] = 5.90\, p = .007, \eta^2 = .53$). Figure 1 displays the mean differences in response latency induced by the different dosages of

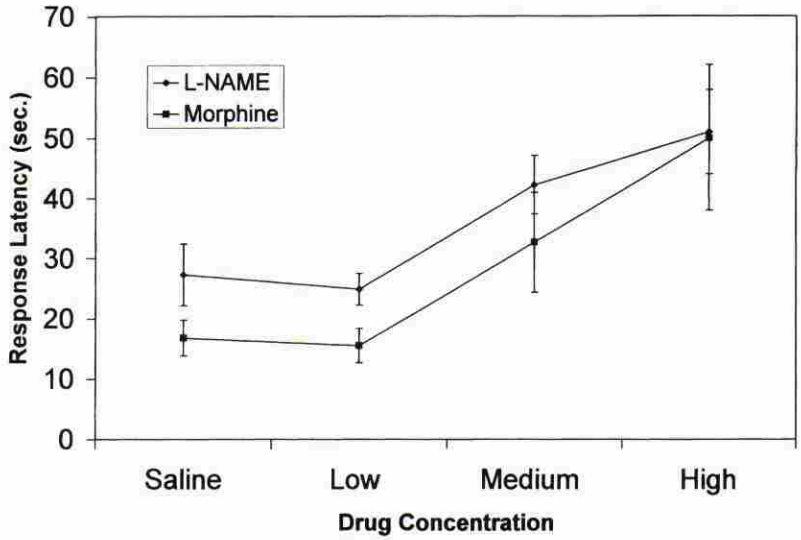

FIGURE 1 Mean response latencies in sec (+/−S.E.M) to thermal stimuli in rats treated with various dosages of L-NAME or morphine. low: 1 mg/kg morphine or 25 mg/kg L-NAME; medium: 4 mg/kg morphine or 50 mg/kg of L-NAME; high: 8 mg/kg morphine or 75 mg/kg L-NAME.

L-NAME. *Post-hoc* one-way analysis of variance demonstrated this main effect could be attributed to the prolongation of response latencies (analgesia) displayed by the group injected with the 75 mg/kg compared to the groups of rats that received either saline, 25 mg/kg or 50 mg/kg of L-NAME; they did not differ significantly from each other. Both the differences in response latencies between the trials ($F[2, 32] = 6.77$, $p = .004$, $\eta^2 = .30$) and between the days ($F[1, 16] = 16.54$, $p = .001$, $\eta^2 = .51$) were statistically significant.

Post-hoc analysis indicated that response latencies on the second and third trial (at 30 min and 60 min) after the treatments were significantly greater than the response latencies during the baseline (trial 1) while the analgesic effect was less on the second day compared to the first day. A statistically significant interaction between trials and days was also detected ($F[2, 32] = 6.96$, $p = .003$, $\eta^2 = .30$). This result is illustrated in Figure 2. Symmetrical *post hoc* analysis indicated that the response latencies (averaged across all drug dosages) on day 1 were significantly elevated at 30 min and 60 min after treatment *versus* these

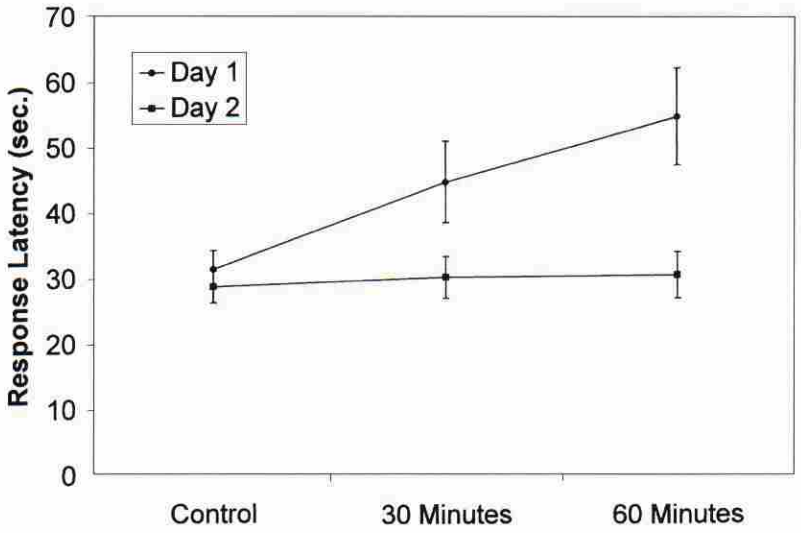

FIGURE 2 Mean response latencies in sec (+/−S.E.M) to thermal stimuli for rats treated with L-NAME as a function of time (trials) for two successive days.

latencies on day 2. Therefore the analgesic potency of L-NAME was evident only on the first day.

Three-way analysis of variance demonstrated a significant difference between the four dosages of morphine ($F[3, 16] = 4.57$, $p = .02$, $\eta^2 = .46$). Figure 1 shows the mean differences in response latencies. *Post-hoc* procedure revealed that the source of the elevated response latencies to thermal stimuli was statistically significant only for the group that had received the 8 mg/kg morphine while the groups that received the other dosages of morphine did not differ from saline-injected controls. Also, significant differences in response latencies existed between the three trials ($F[2, 36] = 13.61$, $p < .001$, $\eta^2 = .46$). *Post hoc* analysis consisting of paired *t*-tests indicated that the significant differences between the daily trials was due to elevated response latencies at 30 min and 60 min compared to the baseline.

The interaction between drug dosage and trial was also significant ($F[6, 32] = 3.70$, $p = .007$, $\eta^2 = .41$). Symmetrical *post-hoc* analysis indicated that the primary source of the difference was due to the elevated response latencies seen in those rats injected with 4 mg/kg and 8 mg/kg of morphine at 30 min *versus* those rats that received either

the saline or 1 mg/kg dosage of morphine (Fig. 3). Unlike the rats that received either the saline or 1 mg/kg dosage, response latencies for the rats that received the two highest dosages were significantly elevated at both 30 min and 60 min relative to baseline levels.

For Experiment II that investigated the potential synergism between these combinations of drugs and acute (30 min) exposures to the complex pulsed magnetic field, four-way analysis of variance demonstrated statistically significant differences in the mean response latencies between the four drug treatments ($F[3, 38] = 26.16$, $p < .001$, $\eta^2 = .67$) but not between the magnetic field *versus* the sham exposed group ($F[1, 38] = 1.99$, $p > .15$). *Post-hoc* one-way analysis of variance indicated that the primary source of differences in response latencies (average of repeated trials and days) between the drug treatments was due to the elevated latencies in the group that received both L-NAME and morphine compared to the saline-injected controls. L-NAME and morphine, administered singly, were found to be significantly more effective than saline in elevating response latencies, but their solitary effects were still significantly less effective than when both drugs were administered together. The differences

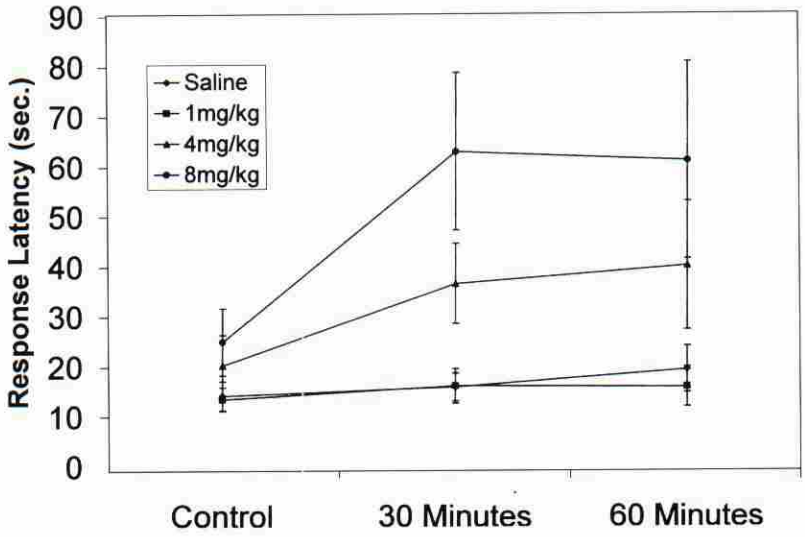

FIGURE 3 Mean response latencies in sec (+/−S.E.M) as a function of time since injection for various dosages of morphine.

between trials was also statistically significant ($F[2, 76] = 59.94$, $p < .001$, $\eta^2 = .61$). Paired t-tests showed that response latencies were significantly greater at 30 min and 60 min post-injection relative to pretreatment (baseline).

The major statistically significant interaction involving the magnetic field exposures was between the drug, field and trial ($F[6, 76] = 2.33$, $p = .04$, $\eta^2 = .16$). These results are shown in Figure 4. The interaction was most parsimoniously explained by the suppression of the analgesia induce by either morphine or L-NAME by the complex magnetic field (in comparison with the sham field-treated group) such that the rats exposed to the complex magnetic field after being injected with either of the two pharmacological analgesics did not differ significantly from saline treated controls either immediately after removal from the field or 30 min after removal from the field (30 min and 60 min after injections). However the magnetic field did not differentially alter the response latencies of rats that had received saline or both morphine and L-NAME relative to the rats exposed to the sham-field. To accommodate for the potential effects of skewness for some of the

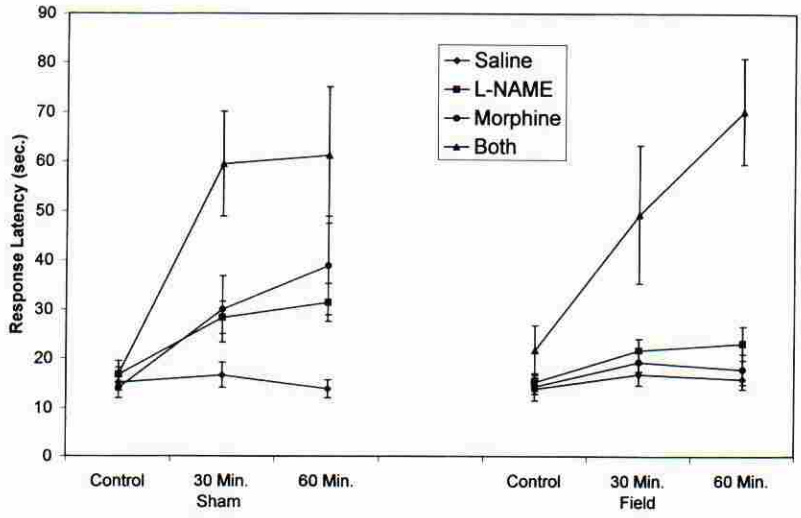

FIGURE 4 Mean response latencies in sec (+/−SEM) response latencies for rats before injections (control) and 30 min later or 60 min later. Between the first and second injections with either saline or one of three drugs (50 mg/kg L-NAME, 4 mg/kg Morphine, 50 mg/kg L-NAME and 4 mg/kg morphine) the rats were exposed for 30 min to either the sham field or the frequency modulated magnetic field.

measurements on some trials, analyses were performed with transformed (log_{10}) data. No alterations in either the directions or the magnitudes of the main effects were observed.

DISCUSSION

We have assumed that the response latency for the criterion response was highly correlated with nociceptive threshold for thermal stimuli applied to the foot pads of rats. The results of these experiments indicated that 30 min exposures to a frequency-modulated magnetic field (the Thomas pattern) that has been shown to produce a pronounced enhancement of analgesia when presented at 100 microTesla to invertebrates (Thomas *et al.*, 1998) attenuated the effects of morphine-induced and L-NAME-induced analgesia to thermal stimuli in normal rats. The effect remained for at least 30 min after the removal of the frequency-modulated magnetic field, suggesting that mediating mechanisms were not due to electrical transients. Whether or not the discrepancy between the results from the present study and the study by Thomas *et al.* (1998) was due to the differences in intensity of the applied field (5 microTesla *vs.* 100 microTesla), the application geometry of the applied field (solenoids *vs.* orthogonal coils), or the animal model (rats *vs.* land snails) must still be discerned.

The complete attenuation of morphine-induced analgesia following exposures to sinusoidal 60 Hz fields or to a 0.5 Hz rotating magnetic fields, whose intensities were more than 100-stronger than those employed in the present study, was reported previously by Kavaliers, Ossenkopp, Prato and Carson (1994). The suppression of L-NAME-induced analgesia by a complex magnetic field, as shown in the present experiment, may be novel. Although Seaman, Belt, Doyle and Mathur (1999) employed an ultrawide electromagnetic field pulsed at 600 times per second in conjunction with 50 mg/kg of L-NAME, statistically significant changes in the nociceptive threshold to thermal stimuli, at least for CF-1 mice, were not observed.

Nitric oxide synthase is responsible for generating nitric oxide (NO) from the amino acid L-arginine in a calcium/calmodulin-dependent manner (Furst, 1999; Stuehr, 1997). According to the mathematical

model first reported by Wolfgang Ludwig (1968), the energies available from the types of complex fields employed in our study would be sufficient to influence the movement of this critical cation and to affect the release of vesicles that generate miniature excitatory post synaptic potentials at the synapse. From this perspective, the attenuation of the analgesic effects would be related to the altered relative concentrations of Ca++. However we cannot exclude the possibility that this particular complex field may have directly affected interactions between chemical radicals or their substrates.

The source of the contradiction between the results reported by Fleming, Persinger and Koren (1994), who employed the same equipment, exposure durations and assessment times as the those in the present study also remains to be explained. Although they presented the magnetic field once every 4 sec the wave pattern was composed of 230 points with 3 msec for each point that comprised a temporal pattern that was designed after the activity of amygdaloid neurons (see Richards, Persinger and Koren, 1993 for a pictorial representation). In the present study a 1 msec pixel for each point that comprised a frequency modulation pattern was employed. If the temporal structure of applied magnetic fields can be considered equivalent to the spatial structure of chemicals, then small changes in the critical temporal parameters of the points that compose a complex magnetic field, like the addition or the removal of methyl groups to and from agonists and antagonists for receptor subtypes, might evoke opposite effects upon the response to pain stimuli.

Acknowledgments

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References

Ferreira, J., Santos, A. R. S. & Calixto, J. B. (1999) The role of systemic, spinal and supraspinal L-arginine-nitric oxide-cGMP pathway in thermal hyperalgesia caused by intrathecal injection of glutamate in mice. *Neuropharmacology*, **38**, 835–842.

Fleming, J. L., Persinger, M. A. & Koren, S. A. (1994) Magnetic pulses elevate nociceptive thresholds: comparisons with opiate receptor compounds in normal and seizure-induced brain-damaged rats. *Electro-and Magnetobiology*, **13**(1), 67–75.

Fürst, S. (1999) Transmitters involved in antinociception in the spinal cord. *Brain Research Bulletin*, **48**(2), 129–142.

Guney, H. Z., Gorgan, C. Z., Tunctan, B., Uludag, O., Hodoglugil, U., Abacioglu, N. & Zengil, H. (1998) Circadian-rhythm-dependent effects of LNG-nitroarginine methyl ester (L-NAME) on morphine induced analgesia. *Chronobiology International*, **15**(3), 283–289.

Kavaliers, M., Choleris, E., Prato, F. S. & Ossenkopp, K.-P. (1998) Evidence for the involvement of nitric oxide and nitric oxide synthase in the modulation of opioid-induced antinociception and the inhibitory effects of exposure to 60-Hz magnetic fields in the land snail. *Brain Research*, **809**, 50–57.

Kavaliers, M., Ossenkopp, K.-P., Prato, F. & Carson, J. J. L. (1994) Opioid systems and the biological effects of magnetic fields, In: Frey, A. H. (Ed.), *On the nature of electromagnetic field interactions with biological systems* (pp. 83–94). Landes: Austin.

Lewis, J. W., Cannon, J. T. & Liebeskind, J. C. (1980) Opioid and nonopioid mechanisms of stress analgesia. *Science*, **208**, 623–625.

Ludwig, H. W. (1968) A hypothesis concerning the absorption mechanism of atmospherics in the nervous system. *International Journal of Biometeorology*, **12**, 93–98.

Machelska, H., Labuz, D., Przelwocki, R. & Prezelwocka, B. (1997) Inhibition of nitric oxide synthase enhances antinociception mediated by mu, delta and kappa opioid receptors in acute and prolonged pain in the rat spinal cord. *Pharmacology and Experimental Therapeutics*, **282**(2), 977–984.

Richards, P. M., Persinger, M. A. & Koren, S. A. (1993) Modification of activation and evaluation properties of narratives by weak complex magnetic field patterns that simulate limbic burst firing. *International Journal of Neuroscience*, **71**, 71–85.

Thomas, A. W., Kavaliers, M., Prato, F. S. & Ossenkopp, K.-P. (1997) Antinociceptive effects of a pulsed magnetic field in the land snail, Cepaea nemoralis. *Neuroscience Letters*, **222**(2), 107–101.

Thomas, A. W., Kavaliers, M., Prato, F. S. & Ossenkopp, K.-P. (1998) Analgesic effects of a specific pulsed magnetic field in the land snail, *cepaea nemoralis*: consequences of repeated exposures, relations to tolerance and cross-tolerance with DPDPE. *Peptides*, **19**(2), 333–342.

Seaman, R. L., Belt, M. L., Doyle, J. M. & Mathur, S. P. (1999) Hyperactivity caused by a nitric oxide synthase inhibitor is countered by ultra-wideband pulses. *Bioelectromagnetics*, **20**, 431–439.

Stuehr, D. J. (1997) Structure-function aspects in the nitric oxide synthases. *Annual Review of Pharmacology and Toxicology*, **37**, 339–359.